

## First Report of *Salmonella enterica* Serotype Panama Meningitis Associated with Consumption of Contaminated Breast Milk by a Neonate

Te-Li Chen,<sup>1</sup> Peck-Foong Thien,<sup>2</sup> Shu-Chin Liaw,<sup>3</sup> Chang-Phone Fung,<sup>1</sup> and L. K. Siu<sup>4\*</sup>

Sections of Infectious Diseases, Department of Medicine, Taipei Veterans General Hospital and National Yang-Ming University, Taipei,<sup>1</sup> Section of Pediatric<sup>2</sup> and Infection Control Committee,<sup>3</sup> I-Lan Hospital, I-Lan, and Infectious Diseases Unit, Division of Clinical Research, National Health Research Institute, Taipei,<sup>4</sup> Taiwan

Received 10 May 2005/Returned for modification 25 June 2005/Accepted 10 July 2005

***Salmonella enterica* serotype Panama is capable of causing severe infection in children and is often transmitted via contaminated food. Here, we present the first documented case of serotype Panama infection that was acquired through the consumption of contaminated breast milk. The mother excreted the organism asymptomatically for at least 2 weeks.**

### CASE REPORT

*Salmonella enterica* serotype Panama is a member of serogroup D1 and is isolated from many foods and animals and from water (3). It generally causes gastroenteritis in humans (12) and is one of several serotypes that tends to cause a disease in children that is more invasive than that caused by other serotypes (13). Cases complicated with bacteremia and meningitis in children have been reported previously (6, 13). The infection is often acquired through the ingestion of contaminated food. This study reports the first documented case of serotype Panama infection acquired via the consumption of contaminated breast milk.

A girl was born vaginally at term with a birth weight of 3,404 g. She was discharged after an uneventful hospitalization for 3 days. On day 13 of the postdelivery period, she exhibited several symptoms, including fever, irritable crying, poor appetite, and watery diarrhea. Her temperature was 38.9°C, blood pressure was 92/63 mm Hg, pulse rate was 152 beats/min, and respiratory rate was 50 breaths/min. Neck stiffness was also noted upon examination.

Laboratory examination revealed a white blood cell count of  $19.5 \times 10^9$  cells/liter with 81% neutrophils, a hemoglobin level of 15.2 g/dl, and a platelet count of  $598 \times 10^9$  cells/liter. The result of urinalysis was normal. Lumbar puncture was performed, and the cerebrospinal fluid (CSF) was yellow in color and slightly turbid in appearance. Analysis of CSF revealed pleocytosis with a white blood cell count of  $3.030 \times 10^9$  cells/liter (neutrophils versus mononuclear cells, 63 versus 37%). The red blood cell count in CSF was  $0.680 \times 10^9$  cells/liter, the protein concentration was 722 mg/dl, and glucose was undetectable. The concomitant plasma glucose level was 145 mg/dl. The Gram's staining of CSF was positive for gram-negative bacilli. The brain echogram revealed no abnormal findings.

*Salmonella* group D1 was concomitantly isolated from blood and CSF samples. The isolates were susceptible to ampicillin (2 µg/ml), trimethoprim-sulfamethoxazole (<0.25/4.75 µg/ml), cefotaxime (<0.5 µg/ml), and ofloxacin (<0.03 µg/ml). The neonate was treated with intravenous cefotaxime at 200 mg/kg of body weight/day for 21 days. Repeated CSF analyses performed before discharge revealed that the CSF was clear in appearance. The white blood cell count in the CSF declined to  $0.098 \times 10^9$  cells/liter, with 88% lymphocytes. The protein and glucose levels were 276 mg/dl and 34 mg/dl, respectively. The Gram stain of that CSF specimen failed to discover bacteria.

The baby was fed entirely with breast milk, either directly or indirectly from freeze-thawed milk that had been stored at 4°C. A sample of frozen milk was sent to the hospital where *Salmonella* serogroup D1 was isolated. Repeated cultivations of breast milk were taken 2 weeks apart and collected by expressing the milk into bottles with a strict aseptic technique at the hospital. An identical serogroup of *Salmonella* was also cultured concomitantly. The mother was aged 34 years, pregnant five times with four births and one abortion. She was robust before and asymptomatic during the perinatal period. She did not exhibit signs of gastroenteritis or mastitis during the observation period and had no history of previous infection. The mother's cultures of the blood and stools were negative for *Salmonella*. The breast milk became sterile after a 7-day course of amoxicillin therapy (500 mg every 8 h). The baby and mother had no history of contact with animals. They lived in a rural area in eastern Taiwan, where they still consumed underground water. Two water samples were collected and sent for bacteriological studies, where coliform levels of 8 and 10 CFU/ml, respectively, were observed. No *Salmonella* was identified. No other family members developed diarrhea during the observation period.

All the *Salmonella* isolates were ribotyped by using an automated Riboprinter microbial characterization system (Qualicon, Wilmington, DE) according to the manufacturer's instructions. The ribotypes of the isolates from the child's blood and CSF and from the mother's breast milk were indistinguishable

\* Corresponding author. Mailing address: Infectious Diseases Unit, Division of Clinical Research, National Health Research Institutes, Rm. 7118, National Defense Medical Center, Taipei 114, Taiwan. Phone: 886 2 26524094. Fax: 886 2 27890254. E-mail: lksiu@nhri.org.tw.

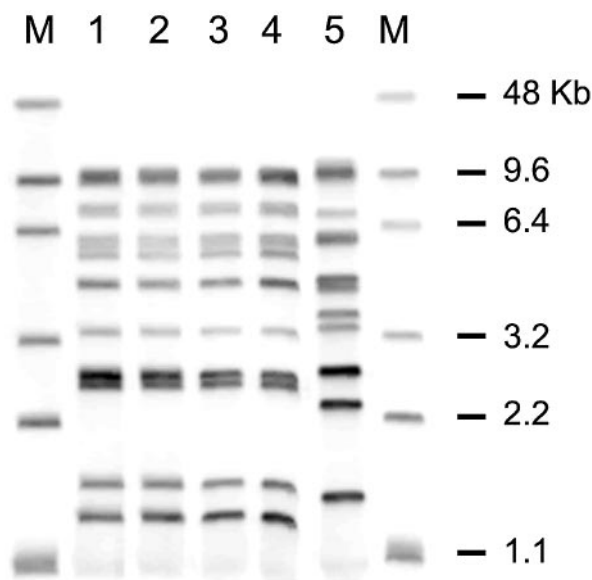


FIG. 1. Ribotyping for serotype Panama isolated from the infant and from breast milk of the mother. Lane 1, isolate from blood; lane 2, isolate from CSF; lane 3 and 4, breast milk isolates from two different periods; lane 5, *Salmonella enterica* serotype Infantis (ATCC 51741). M, marker lane.

(Fig. 1). The organisms were identified as serotype Panama after comparing the banding patterns with the database in the Riboprinter system. The pulsed-field gel electrophoresis study showed a smear after restriction enzyme digestion (data not shown).

The infant suffered from recurring *Salmonella* meningitis after discharging for 2 weeks, even though she was not fed with breast milk during this period. The stool, blood, and CSF cultures displayed the presence of *Salmonella* serogroup D1. Antimicrobial therapy with intravenous cefotaxime at 200 mg/kg/day was administered. Upon controlling the infection, the baby was transferred to a medical center, where a ventricular-peritoneal shunt was implanted for the obstructive hydrocephalus that was detected by a computed tomography scan.

**Discussion.** For infants, human milk is considered a food superior to formula food. Besides having a nutritional advantage, it also has long-term benefits for metabolism and for disease prevention in later life. It also helps in protecting against infections through specific and nonspecific immune factors (7). A case control study showed that breast-feeding decreased the risk of sporadic salmonellosis in infants (10). However, breast milk has also been implicated as the source of several viral and bacterial infections in neonates (8, 9), including that from salmonellae. One serotype, *Salmonella enterica* serotype Kottbus, was initially suggested to have a specific predilection for colonizing the human mammary gland (1, 11). In the following years, several reports showed that other serotypes, including *Salmonella enterica* serotype Typhimurium (4), *Salmonella enterica* serotype Typhimurium definite type 104 (DT104) (8), and *Salmonella enterica* serotype Senftenberg (9), can also be transmitted via breast milk. Our report extended

the list of salmonellae that might be transmitted via this vesicle.

In this case, the repeated isolation of serotype Panama from milk collected aseptically strongly suggested that the mother is the carrier who excreted this pathogen into breast milk rather than the possibility that the milk was contaminated during collection. The excretion of *Salmonella* organisms from the breast asymptotically is not unique to this case (1, 9). However, the source from and period within which the mother became colonized with this organism could not be traced and remain unknown. The *Salmonella* organisms could enter into the mammary duct via external contamination. Qutaishat et al. suggested that the priming of *Salmonella* organisms into the mammary gland via macrophage, after the ingestion of the organisms via the gastrointestinal tract, could also be a possible cause of pathogenesis (8). Our results showed that once serotype Panama colonizes the mammary duct, the organism can be excreted from the breast for at least 2 weeks before treatment is initiated. According to a study performed by Qutaishat et al., the gene fragment of *Salmonella enterica* serotype Typhimurium DT104 could be detected in contaminated breast milk for eight consecutive days by using a semiquantitative PCR (8). In that case, the mother also received antibiotic therapy, but the timing of its initiation was not shown.

Since the infant was fed with breast milk only, the contaminated milk is considered the source of infection, and the possibility that the pathogen was acquired from other sources is discounted. However, no quantitative study of the bacteria in the contaminated milk was performed. Hence, it is not known whether the infection had been developed via direct nursing or through the consumption of freeze-thawed milk. There are cases reported to be infected via direct nursing by a mother with (5) or without (9) mastitis. Nevertheless, breast milk that was improperly collected and left in room temperature for a prolonged period before storage has accounted for several *Salmonella* outbreaks in neonatal intensive care units (1, 4, 9). Therefore, the use of a correct procedure for the collection and storage of breast milk is crucial for preventing the bacteria from proliferating to an infectious level. The fact that the bacteria could be isolated from thawed milk suggests that once the milk gets contaminated with a certain amount of serotype Panama, it remains a potential source for transmission of the organism, even though it had been stored at 4°C.

Macrorestriction of chromosomal DNA followed by pulsed-field gel electrophoresis is considered a gold standard in epidemiologic studies of diverse bacterial species. However, this method cannot be applied to serotype Panama, because the organism's intrinsic nucleus activity would result in the degradation of its DNA during processing (12). On the contrary, the ribotyping of this species has been previously performed successfully (12). Advantages in automation and standardization make the Riboprinter microbial characterization system suitable for rapid and high-throughput typing of bacterial strains (2). Our results show that automated ribotyping is an applicable method for typing serotype Panama.

Serotype Panama infection in neonate might be acquired through contaminated breast milk. Once it colonizes the breast, serotype Panama might be shedding from the breast for at least 2 weeks before treatment is initiated.

This work was supported by an internal grant from the National Health Research Institutes.

# REFERENCES

1. **Anonymous.** 1978. Nosocomial salmonellosis in infants associated with consumption of contaminated human milk: summary of two outbreaks. *Natl. Nosocomial Infect. Study Rep.* **1978**:14–15.
2. **Brisse, S., D. Milatovic, A. C. Fluit, K. Kusters, A. Toelstra, J. Verhoef, and F. J. Schmitz.** 2000. Molecular surveillance of European quinolone-resistant clinical isolates of *Pseudomonas aeruginosa* and *Acinetobacter* spp. using automated ribotyping. *J. Clin. Microbiol.* **38**:3636–3645.
3. **Cordano, A. M., and R. Virgilio.** 1996. Evolution of drug resistance in *Salmonella panama* isolates in Chile. *Antimicrob. Agents Chemother.* **40**:336–341.
4. **Drhova, A., V. Dobiasova, and M. Stefkovicova.** 1990. Mother's milk—unusual factor of infection transmission in a salmonellosis epidemic on a newborn ward. *J. Hyg. Epidemiol. Microbiol. Immunol.* **34**:353–355.
5. **Gibb, A. P., and P. D. Welsby.** 1983. Infantile salmonella gastroenteritis in association with maternal mastitis. *J. Infect.* **6**:193–194.
6. **Kostiala, A. A., M. Westerstrahle, and M. Muttillainen.** 1992. Neonatal *Salmonella panama* infection with meningitis. *Acta Paediatr.* **81**:856–858.
7. **Oddy, W. H.** 2001. Breastfeeding protects against illness and infection in infants and children: a review of the evidence. *Breastfeed. Rev.* **9**:11–18.
8. **Qutaishat, S. S., M. E. Stemper, S. K. Spencer, M. A. Borchardt, J. C. Opitz, T. A. Monson, J. L. Anderson, and J. L. Ellingson.** 2003. Transmission of *Salmonella enterica* serotype Typhimurium DT104 to infants through mother's breast milk. *Pediatrics* **111**:1442–1446.
9. **Revathi, G., R. Mahajan, M. M. Faridi, A. Kumar, and V. Talwar.** 1995. Transmission of lethal *Salmonella senftenberg* from mother's breast-milk to her baby. *Ann. Trop. Paediatr.* **15**:159–161.
10. **Rowe, S. Y., J. R. Rocourt, B. Shiferaw, H. D. Kassenborg, S. D. Segler, R. Marcus, P. J. Daily, F. P. Hardnett, and L. Slutsker.** 2004. Breast-feeding decreases the risk of sporadic salmonellosis among infants in FoodNet sites. *Clin. Infect. Dis.* **38**(Suppl. 3):S262–S270.
11. **Ryder, R. W., A. Crosby-Ritchie, B. McDonough, and W. J. Hall III.** 1977. Human milk contaminated with *Salmonella kottbus*. A cause of nosocomial illness in infants. *JAMA* **238**:1533–1534.
12. **Stanley, J., N. Baquar, and A. Burnens.** 1995. Molecular subtyping scheme for *Salmonella panama*. *J. Clin. Microbiol.* **33**:1206–1211.
13. **Yang, Y. J., M. C. Huang, S. M. Wang, J. J. Wu, C. P. Cheng, and C. C. Liu.** 2002. Analysis of risk factors for bacteremia in children with nontyphoidal *Salmonella* gastroenteritis. *Eur. J. Clin. Microbiol. Infect. Dis.* **21**:290–293.